

# Development of a pharmacodynamic model for HIV treatment with nucleoside reverse transcriptase and protease inhibitors

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Received 11 February 2002; accepted 21 June 2002

## Abstract

There is a need for models useful for predicting the efficacy of agents developed for treating human immunodeficiency virus (HIV) based on information obtained during the drug development process. A pharmacodynamic model that superimposes the pharmacokinetics of anti-HIV nucleoside reverse transcription (RT) and protease inhibitors over a previously published predator–prey model of HIV and CD4 dynamics was developed to address this need. This model was applied to in vitro measurements and patient-derived pharmacokinetics of the unbound antiviral drugs to simulate HIV-1 and CD4 counts versus time and dose. The primary mechanism for nucleoside RT inhibitors was assumed to be competitive inhibition of HIV-1-RT by the active nucleoside triphosphates (NTP). Cellular accumulation and egress rates of the NTP were estimated from previous in vivo pharmacokinetic studies. Median inhibition concentrations for the HIV-1 RT enzyme were estimated from previously published cell-free binding studies. The concentration of active protease inhibitor available for binding with HIV-1 protease was assumed equal to the unbound fraction in the plasma. The resulting simulations for mono- and dual nucleoside therapy with zidovudine and lamivudine single dose regimen with the protease inhibitor indinavir, produced similar HIV and CD4 response profiles to those reported in large Phase II and III clinical trials. Based on these findings this pharmacodynamic model can be applied to predict starting doses for a new agent based on simulated biological responses as a function of time for dosage regimens comprising one or two agents. However, the model overestimated the efficacy of highly effective drug combinations where all three agents are combined as in highly active anti-retroviral therapy. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** HIV dynamics; Pharmacokinetics; Pharmacodynamics

## 1. Introduction

Patients treated with combinations of anti-HIV drugs have a greater prognosis than those treated with monotherapy regimens (Rutchmann and

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Hirschel, 1997). The most common mechanisms for anti-HIV drugs used in the clinic are inhibition of HIV reverse transcription (RT) by incorporating nucleoside triphosphate (NTP) (such as zidovudine-triphosphate [ZDV-TP] and lamivudine-triphosphate [3TC-TP]) in elongating cDNA chains or by inhibiting protease virus assembly (e.g. indinavir).

Clinical trials are required to demonstrate the efficacy of new drug regimens before they are accepted in clinical practice. However, these studies are often expensive and time consuming. Clinical trials are usually designed to randomize many individuals to cover a wide range of doses, especially when starting doses are not optimized. Although the Food and Drug Administration (FDA) only requires two pivotal efficacy studies in support of new drug applications (NDA), the average NDA requires many more. Almost 50% of all studies did not reach statistical significance for various reasons, most relating to the design of the study (Bonate, 2000). In silico simulations as predictive tools are becoming increasingly popular methods for improving the design of clinical trials of novel drug candidates and for eliminating compounds early in the development phase. This paper provides an overview of a predictive model specifically for HIV nucleoside RT and protease inhibitors.

According to the current ‘Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents’ (Fauci et al., 2000), infected patients should not be treated with drug regimens that are less effective than those approved for highly effective antiretroviral therapy (HAART) combinations. Therefore, it is unethical to commence efficacy studies using monotherapy with new agents, especially if these agents were developed to replace individual components in existing HAART regimens. Since monotherapy with a novel agent for a prolonged period is no longer permitted due to the potential development of resistant HIV, investigators may have to select a starting dose for a novel drug component of HAART based on existing preclinical and Phase I data. The utility of these data to select a dose no

less potent than the agent it may be designed to replace may be enhanced through pharmacodynamic modeling.

Preclinical pharmacology studies on anti-HIV agents are performed early in the drug development process and include cell free assays to characterize the inhibition with various viral enzymes, drug metabolism in cell culture, and the inhibition of virus replication in infected cells. Furthermore, pharmacokinetic data are available from animal studies and Phase 1 clinical studies before efficacy studies are initiated.

Mathematical modeling has contributed much to our understanding of the dynamics of HIV and target CD4 cells in HIV infected patients (Perelson et al., 1996; Bonhoefer et al., 1997; Novak et al., 1997; Cavert et al., 1997; Novak and May, 2000; Nelson et al., 2000; Ramratnam et al., 2000; Stafford et al., 2000). Most existing models of viral dynamics during HIV treatment have concentrated on determining the degree of virus suppression ( $\rho$ ) using patient virus loads (Bonhoefer et al., 1997; Novak et al., 1997; Cavert et al., 1997). However, few models exist that relate  $\rho$  to in vivo pharmacokinetics and pharmacodynamic parameters, such as binding constants to viral RT and protease targets or other pharmacological parameters measured in preclinical pharmacology studies. Therefore, there is a need for models that can optimize the utility of all available information by merging data from pharmacological testing with existing in vivo HIV-1 dynamics models. Such models could help determine whether potential drugs warrant further testing in the clinic and may suggest starting dose regimens for future efficacy studies.

A model was developed that relates the HIV dynamics to drug doses and pharmacokinetics and antiviral pharmacodynamic parameters. ZDV, 3TC and indinavir were chosen as model drugs for this study since they are representative of nucleoside RT and protease inhibitors that are commonly included in modern HAART regimens. In addition, published data from large clinical trials with these drugs are available for comparison with model predictions.

## 2. Methods

### 2.1. Biological considerations

The most widely used model of HIV dynamics in infected patients considers the infection as an interaction of a predator (HIV) and its prey (CD4 positive lymphocytes) (Perelson et al., 1996; Bonhoefer et al., 1997; Novak et al., 1997; Novak and May, 2000; Nelson et al., 2000; Stafford et al., 2000), although other mechanisms of pathology have also been proposed (Cloyd et al., 2000). Stafford et al. (2000) demonstrated that the predator versus prey model, when fit to the HIV and CD4 profiles of newly infected patients, fully described the out of phase ‘oscillations’ in CD4 cell counts in blood and HIV loads during the first 4 months of infection.

The overall pharmacodynamic model proposed for HIV-1 during treatment with nucleoside RT inhibitors and protease inhibitors is shown in Fig. 1. This model superimposed drug effects over the predator prey model. Rate constants for cell and virus dynamics were adapted from the literature to ensure that estimates were physiologic (see Table 2; Nelson et al., 2000; Perelson et al., 1996; Stafford et al., 2000). Initial estimates selected

from the literature for infected and uninfected CD4 cells and HIV loads appeared relatively stable for simulations over a period of days. However, periodic fluctuations of HIV loads and CD4 counts were evident in simulations of data points lasting weeks to months. Therefore, pre-drug starting HIV loads and CD4 counts were obtained by permitting a theoretical simulation to occur until HIV and CD4 levels converged on values that were stable over a simulation period that lasted for an additional 4 months. These equilibrium baseline HIV loads were in the high range ( $\sim 1.35 \times 10^6/\text{ml}$ ), and the CD4 counts were in the lower range of those observed in the clinic ( $\sim 18$  cells per  $\mu\text{l}$ ) (Table 2). This may be the result of infections not reaching ‘true equilibrium’ condition in most patients. Equations of Stafford et al. (2000), (equations 5–7) were adapted to include drug effects (see below).

### 2.2. Pharmacokinetic model with separate effect compartment biophase

By assuming that each virus level was related to the previous virus load, CD4 count, and drug levels, simultaneous differential equations were used to link drug concentrations in the plasma to

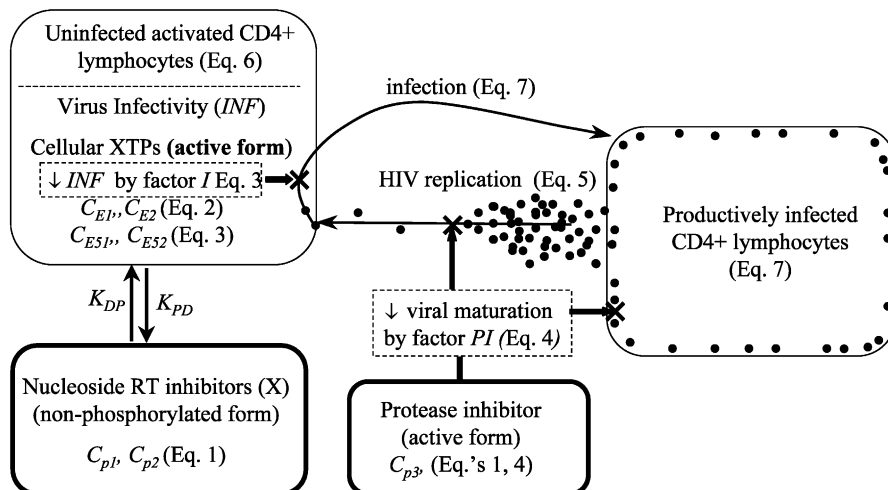


Fig. 1. Overall schematic representation of the pharmacodynamic model for HIV-1 describing the conversion of RT inhibitors (X) to their respective active NTP metabolites (XTPs) in uninfected cells. The accumulation of XTPs results in a decrease in the infectivity of HIV (INF) by a concentration dependent factor ( $I$ ). Also shown, is the interaction of a protease inhibitor, which causes a decrease in the maturation of HIV particles by a concentration dependent factor (PI) (please refer to text).

the inhibition of infection of CD4 cells. Since anti-HIV therapy is prolonged (months to years), it was assumed that pseudo-steady-state plasma concentrations, defined by repeatable troughs and peaks in plasma concentrations between doses, would occur relatively early in treatment. Therefore, efficacy should be related to the average plasma drug concentration corrected for protein binding, calculated using the formula:  $C_{p, \text{ average}} = \text{AUC} / (\text{dose interval})$ , (Equation 1), where AUC is the single-dose area under the plasma concentration of unbound drug versus time curve from time 0 to infinity, and is equivalent to the AUC value between each dose interval once steady state has been reached (Gibaldi and Perrier, 1982). The use of an average plasma concentration is especially warranted for nucleoside RT inhibitors like ZDV and 3TC, where the nucleosides are phosphorylated to nucleotides and degraded slowly in cells. However, it may be less accurate for protease inhibitors such as indinavir, which do not require metabolic activation and where equilibration between plasma and cellular concentrations drug is expected to be rapid.

The effect of nucleoside RT inhibitors was assumed to result from the accumulation of active nucleotide triphosphate (NTP,  $C_E$ ) that accumulates in cells. Although the dynamics of accumulation and breakdown of the NTP are more complex than simple first order processes, these processes were approximated as first order in Equation 2:

$$\frac{dC_{E1}}{dt} = K_{DP1} C_{p1} - K_{PD1} C_{E1} \quad \text{and}$$

$$\frac{dC_{E2}}{dt} = K_{DP2} C_{p2} - K_{PD2} C_{E2}$$

Very few data are available on the accumulation and egress of NTPs in vivo. However, published patient data were available to estimate the NTP accumulation and breakdown rates ( $K_{DP}$  and  $K_{PD}$ , NTP, respectively) for lamivudine and ZDV. In the case of ZDV and lamivudine, breakdown rates measured in vitro using human peripheral blood mononuclear (PBM) and the lymphoblastoid CEM cells produce similar results to isolated PBM cells of patients (Solas et al., 1998). However, it has been reported that Abacavir, a nucleo-

side prodrug that undergoes a complex anabolic pathway to form the active carbovir-triphosphate (CBVTP), the intracellular  $t_{1/2}$  of CBVTP is 3 h, while in patient PBM cells it is  $> 20$  h (Daluge et al., 1997; Faletto et al., 1997; Parker et al., 1993; Kewn et al., 2002). This suggests that egress rate constants measured in PBM cells should be considered more reliable than from CEM cells. Calculations of ZDVTP from plasma concentrations using equation 2 are also an oversimplification, since cellular ZDVTP and plasma ZDV may not be linearly related to dose (Barry et al., 1996). Nonlinearity in accumulated ZDVTP levels may result from a rate limiting monophosphorylation by thymidine kinase (TK), or from other indirect mechanisms, such as the susceptibility to removal of the chain terminating ZDV-monophosphate from the 3'-terminus of the primer from through enzymatic pyrophosphorolysis catalyzed by HIV-1-RT (Lavie et al., 1997; Arion and Parniak, 1999). The  $C_E$  of ZDVTP used in this study was calculated from the average concentration measured in the lymphocytes of patients given 150 mg twice daily. This aspect of the model could be refined once more detailed cellular enzymatic data becomes available for ZDV.

The fraction of RT inhibited by NTP =  $I$  (see below). Nucleoside agents are usually used in combinations comprising a TK dependent nucleoside agent, (e.g. ZDV) and a TK independent nucleoside RT inhibitor, (e.g. 3TC). Since the NTP compete for the same RT enzyme,  $I$  was modeled using equation 3 (Gabrielson and Weiner, 1997):

$$I = 1 - \frac{[I_{\max 1} [C_{E1}/C_{E51}]^{\eta_1} + I_{\max 2} [C_{E2}/C_{E52}]^{\eta_2}]}{[1 + C_{E1}/C_{E51}]^{\eta_1} + [C_{E2}/C_{E52}]^{\eta_2}}$$

Protease inhibitors like indinavir do not require metabolism to an active species. Therefore, it was assumed that the average non-protein bound fraction in the plasma ( $C_{p3}$ ) equilibrates rapidly with the concentration of drug contained in the cellular compartment where virus maturation occurs ( $C_{p3} = C_{E3}$ ).  $C_{p3}$  may differ considerably from the average plasma concentration calculated using equation 1, especially if the dosing interval ( $\tau$ ) is prolonged. This may result in a between dose trough value of  $C_{p3}$  sufficiently low to allow virus

Table 1  
Drug related parameters (please refer to text for sources)

Drug	ZDV	3TC	Indinavir
Daily oral dose (mg)	300 bid	150 bid	800 mg tid
DailyAUC ( $\mu\text{M}$ )	$3.82 \times 10^3$	$1.84 \times 10^3$	$3.04 \times 10^3$
Free fraction in serum	0.64	0.64	0.20
$K_{\text{DP}}$ (per day)	0.66	4.75	NA
$K_{\text{PD}}$ (per day)	2.14	1.11	NA
$\text{IC}_{50}$ ( $\mu\text{M}$ )	0.035 (a)	0.65 (b)	0.08 (c)
Exponent ( $\eta$ )	Assumed = 1	1.32	Assumed = 1

AUC1, AUC2, AUC3 are the cumulative areas under the plasma concentration vs. time curves/day for ZDV, 3TC and indinavir, respectively. The dose intervals ( $\tau$ ) were assumed to be 12 hourly for b.i.d., and 8 hourly for t.i.d.  $K_{\text{DP1}}$ ,  $K_{\text{DP2}}$  and  $K_{\text{PD1}}$ ,  $K_{\text{PD2}}$  are the respective accumulation/egress rate constants for ZDV and 3TC.  $\text{IC}_{50}$  ( $\mu\text{M}$ ) are concentrations required for 50% inhibition of the target enzyme of HIV-1. (a) ZDVTP vs. the RT of cloned HIV-1 (Reardon and Miller, 1990). (b) 3TCTP vs. the RT of strain xxBRU (Lerma et al., 1999). (c) Indinavir vs. HIV-1 infected PBM cells (St. Clair et al., 1996; Winslow et al., 1994).  $\eta$  is the concentration exponent that permits the model to describe dose-response relationships that are not directly proportional to drug concentration. By considering in vitro dose-response data, it was assumed that at sufficient concentration each drug should be able to completely inhibit virus replication ( $I_{\text{max}} = 1$ ).

maturation to proceed, and a recovery in infectious virus loads. Therefore, both AUC and  $\tau$  are important prognostic indicators of treatment efficacy with HIV protease inhibitors (Anderson and Fletcher, 2001). Provided  $\tau$  remains constant and pharmacokinetics remain linear, the AUC is proportional to the between dose trough concentrations. For simplicity, it was assumed that  $\tau$  was sufficiently small, so that drug efficacy would be proportional to average plasma concentrations, allowing the inhibition of viral protease (PI) to be modeled using equation 4:

$$\text{PI} = 1 - \frac{[I_{\text{max}3} C_{\text{p}3}^{\eta_3}]}{[C_{\text{p}53}^{\eta_3} + C_{\text{p}3}^{\eta_3}]}$$

Model estimates for  $I$  and PI versus time and dose were superimposed over a previously published model of HIV and CD4 dynamics (equation 5–7) (Stafford et al., 2000). The change in viral load 3 was modeled using equation 5:

$$\frac{dV}{dt} = \text{CD4}_{\text{inf}} \times \text{prod}[\text{PI}] - KV$$

In equation 5, prod is the average rate of virus output per infected CD4 cell (see below).

The dynamics of uninfected CD4 cells was modeled using equation 6:

$$\frac{d\text{CD4}}{dt} = R_0 - K_{\text{un}}\text{CD4} - \text{INF} \times V \times \text{CD4}[I].$$

The infected CD4 cells are described by equation 7:

$$\frac{d\text{CD4}_{\text{inf}}}{dt} = \text{INF} \times V \times \text{CD4}[I] - K_{\text{inf}}\text{CD4}_{\text{inf}}$$

In equation 6 and 7 INF is the second order constant relating rate of infection of CD4 cells to  $V$ . The symbols representing the drug and biological related parameters are defined in the legends of Tables 1 and 2, respectively.

Simulations were performed with the aid of a differential equation fitting and simulation program (WINNONLIN ver. 1.5, 1997. Scientific Consulting, Inc., Carey, NC). Estimates of the drug-related and biological parameters used in the model are summarized in Tables 1 and 2, respectively.

### 2.3. Plasma pharmacokinetics

Human plasma pharmacokinetic parameters are usually available before efficacy studies are conducted in the clinical drug development process. HIV drugs are administered over a prolonged period (month to years). Pharmacokinetic steady-state occurs when peak and trough plasma con-



Table 2

Biological parameters describing cell and virus dynamics (obtained from Nelson et al., 2000; Perelson et al., 1996; Stafford et al., 2000)

<i>Cell Dynamics</i>	
Input rate of uninfected CD4+ cells ( $R_0$ )	5 per $\mu\text{l/day}$
Maximum CD4+ population size	$1.5 \times 10^3/\mu\text{l}$
Death rate of infected cells	0.39/day
Death rate of uninfected cells	0.01/day
Division rate of uninfected cells	0.03/day
Median survival of infected cell	2.6 day
Second order infection rate constant (INF)	$6.5 \times 10^{-4}$
<i>Virus Dynamics</i>	
Burst size of infected cells	850 copies per cell
Elimination rate constant of virions	3.07/day
<sup>a</sup> Pseudo-equilibrium start conditions for simulations	
Uninfected cells/ $\mu\text{l}$	5.6
Productively infected cells/ $\mu\text{l}$	12.7
HIV copies/ml serum	$1.35 \times 10^6$

$(dV/dt)(dCD/dt)(dCD_{4\text{inf}}/dt)$  are the rates of change in virus load and CD4 infected and uninfected cells, respectively.  $V$  is the virus load/ml serum.  $CD_4$  and  $CD_{4\text{inf}}$  are the uninfected and infected CD4 cells/ $\mu\text{l}$  prod is the average number of HIV copies produced per infected cell/day.  $R_0$  is the input rate of uninfected CD4 cells/day per  $\mu\text{l}$  of blood and  $K$  is the first order elimination rate constant for HIV from blood. INF is a second order rate constant relating infection rates to HIV and CD4 cells (infection rate =  $\text{INF} \times \text{CD4 cells}/\mu\text{l} \times \text{virus load}/\text{ml}$ ).  $K_{\text{inf}}$  and  $K_{\text{un}}$  are first order elimination rate constants for infected and uninfected cells, respectively.

<sup>a</sup> The model converged on these parameters when drug concentrations were set = 0 and allowed to simulate virus loads and CD4 cells until no further fluctuations were observed (see text).

centrations become repeatable between doses and is reached early in treatment, taking four to five terminal half-lives. Once steady-state is reached the total amount of drug entering the blood plasma during one dosing interval equals the amount of drug eliminated from the plasma. The drug area under the plasma concentration time curve (AUC) between doses is equal to the area under the AUC value obtained from a single-dose pharmacokinetic study. Therefore, the average plasma concentration equals AUC divided by the dose interval (equation 1) (Gibaldi and Perrier, 1982). Since only unbound drug is free to enter

cells, the average plasma concentration of unbound drug ( $C_{p1}$ ,  $C_{p2}$  and  $C_{p3}$ ) was obtained by multiplying this value by the fraction of drug not bound to proteins. (see Table 1). AUC values and fraction of drug bound to protein were taken from the literature (Haas et al., 2000; Package insert 1, 2002; Package insert 2, 2002; Package insert 3, 2002).

#### 2.4. Concentrations of ZDV and 3TC triphosphates in the 'effect biophase'

Susceptible cells are protected from HIV by the accumulation of NTPs. Calculation of the concentration in this compartment (CE) requires estimates of the cell volume together with accumulation ( $K_{\text{DP}}$ ) and breakdown ( $K_{\text{PD}}$ ) rate constants.  $K_{\text{DP}}$  and  $K_{\text{PD}}$  were calculated from data obtained in vitro or in vivo. Accumulation and egress data of ZDV and 3TC were available from pharmacokinetic studies in patients (Barry et al., 1996; Solas et al., 1998). In our model,  $K_{\text{PD}}$  values were estimated from a plot of  $\ln(\text{pmol NTP per } 10^6 \text{ cells})$  versus time.  $K_{\text{DP}}$  values were then estimated from pseudo-equilibrium levels of NTP reported in these papers. NTP levels were reported in units of pmol per  $10^6$  PBM cells. Cellular concentrations of NTP ( $C_E$ ) were calculated by assuming a CD4 cell volume of  $1.1 \mu\text{l per } 10^6 \text{ cells}$ , the volume previously measured for CEM cells (Hurwitz et al., 2000). Average plasma concentrations of unbound drug were estimated as described above.  $K_{\text{DP}}$  values were then calculated by assuming rates of accumulation and egress of the NTP in cells are at equilibrium. (i.e.  $C_p \times K_{\text{DP}} \times C_p = K_{\text{PD}} \times C_E$ ) (equation 8) (see Table 1). For ZDV (600 mg/day) and 3TC (300 mg/day), the predicted average concentrations of NTP in the 'effect biophase' following dose equilibration were 0.075 and 5.04  $\mu\text{M}$ , respectively.

#### 2.5. Viral pharmacodynamic parameters of active drugs $CE_{50}$ and $\eta$ -values

The median effective concentrations for ZDV and 3TC active triphosphate ( $EC_{50}$  values) versus HIV-1 RT were obtained from cell-free enzyme inhibition studies that utilized isolated enzyme

(Lerma et al., 1999; Reardon and Miller, 1990).

The sensitivity of the pharmacological response to dilution (concentration exponents  $\eta_1$  and  $\eta_2$ ) was estimated from a log–log plot of active NTP versus percent enzyme inhibited (see Table 1). The predicted cellular concentrations of the NTP were used to calculate inhibition of HIV-1 RT ( $I$ ) as a function of time.

The effect of the NTP on HIV-1 RT was modeled by multiplying the infectivity rate constant by equation 4. This equation models the additive competitive interaction of  $C_{E1}$  and  $C_{E2}$  (equations 6 and 7 of model).

The median effect concentration of indinavir ( $C_{p53}$ ) was obtained from in vitro studies of protease inhibition in infected human PBM cells (St. Clair et al., 1996; Winslow et al., 1994). The concentration exponent,  $\eta_3$  was estimated as 1 (see Table 1). The average plasma concentration of the protease inhibitor was used to predict inhibition of HIV-1 protease (PI). The prod parameter was then multiplied by PI (equation 5 of model).

### 3. Results

The simulated  $\log_{10}$  reduction in virus load and CD4 counts/ $\mu$ l, versus weeks of treatment are contained in Figs. 2 and 3, respectively. The respective curves are ZDV (600 mg/day, curve 1), 3TC (300 mg/day, curve 2), combined therapy (600 mg ZDV and 300 mg 3TC/day, curve 3), indinavir (2400 mg/day, curve 5) and indinavir together with ZDV (600 and 1200 mg/day, curve 5). These predictions were compared with previously reported data from actual clinical trials (Montaner et al., 1998; Gulick et al., 2000; Hirsch et al., 1999; Package insert 3, 2002).

### 4. Discussion

The pharmacodynamic model was developed to link pharmacokinetic plasma concentration profiles of single or dual combinations of antiretroviral drugs with median pharmacodynamic

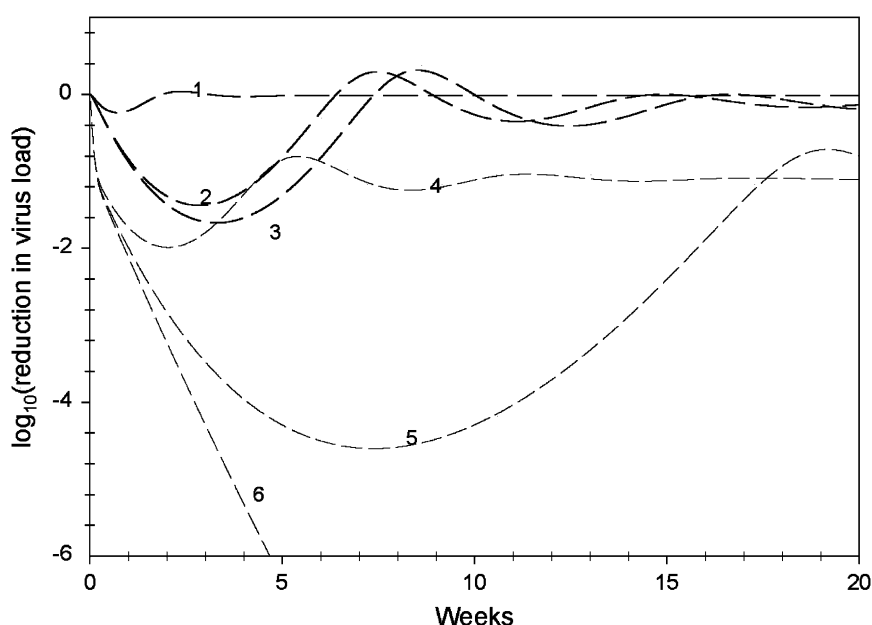


Fig. 2. Predicted HIV loads in serum vs. time for patients given ZDV 600 mg/day (curve 1), lamivudine 300 mg/day (curve 2), ZDV together with lamivudine (curve 3), indinavir 2400 mg/day (curve 4), indinavir 2400 mg/day together with ZDV 600 mg/day (curve 5), and indinavir 2400 mg/day together with lamivudine 300 mg/day (curve 6). Most current assays are limited in their ability to detect virus loads < 400 counts per ml.

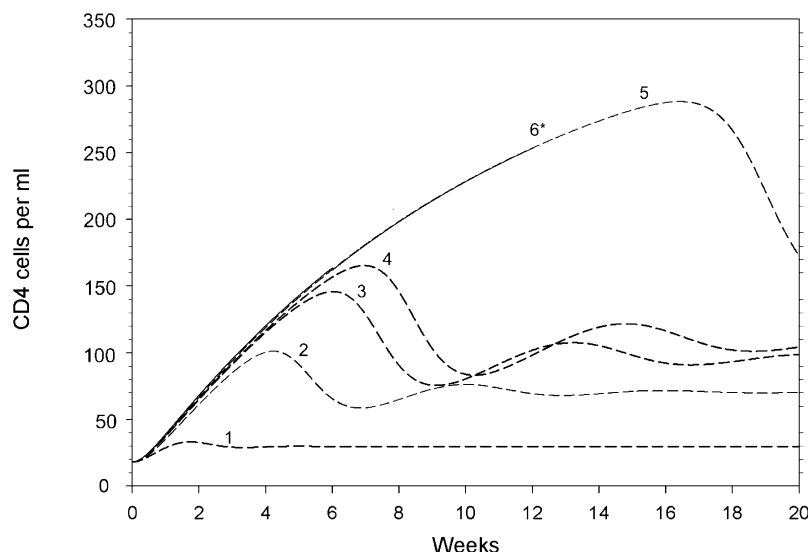


Fig. 3. Predicted CD4 cell counts (infected+uninfected cells) in blood vs. time for patients given ZDV 600 mg/day (curve 1), lamivudine 300 mg/day (curve 2), ZDV together with lamivudine (curve 3), indinavir 2400 mg/day (curve 4), indinavir 2400 mg/day together with ZDV 600 mg/day (curve 5), and, indinavir 2400 mg/day together with lamivudine 300 mg/day (curve 6). \*Curve 6 was simulated only until HIV load depletions were  $< 6$  logs (please refer to text).

parameters, such as cellular accumulation rate constants and  $EC_{50}$  values versus target enzymes, to predict median trends in HIV loads and CD4 counts. The present model does not consider the substantial variations in drug levels (pharmacokinetics or medication adherence) and virus susceptibility (pharmacodynamics), that may exist within patient populations (Anderson and Fletcher, 2001; Barry et al., 1996; Sommadossi, 1998; Moore et al., 2000; Rodriguez et al., 2000; Pellegrin et al., 2001; Kakuda et al., 2001; Kewn et al., 2002). However, the equations presented could be adapted for use with nonlinear mixed effects software to predict the variance in patient virus loads and CD4 counts, once the variance in pharmacokinetic and viral susceptibilities become available (Sheiner and Beal, 1980; Mandema et al., 1992).

Periodic fluctuations in HIV loads and CD4 counts were observed when starting values of HIV loads and CD4 cell counts were taken directly from the literature. To stabilize the oscillations so that all further fluctuations would be due to the drug therapy, the program was permitted to converge on model estimates for CD4 cell counts and HIV loads, such that the equations predicted

steady-state virus loads and CD4 cells counts lasting for an additional 4 months in the absence of drug therapy (baseline conditions). These resulting baseline HIV loads were higher ( $\sim 1.35 \times 10^6$  copies per ml), and the CD4 counts were in the lower range ( $\sim 20$  cells per  $\mu$ l) than those most commonly observed in treatment naive patients ( $\sim 10^5$  copies per ml and  $\sim 180$  cells per  $\mu$ l, respectively) (Kaufman et al., 2000). Since viral load reduction is related to the starting virus load, a larger pretreatment virus load may require more time (and/or drug) to reach levels below the limit of detection ( $< 400$  copies per ml) than for patients with lower starting virus loads (Cohen and Fauci, 2000). A higher pretreatment viral load may also predict a greater treatment time before the virus reaches an undetectable level. The model does not consider drug toxicity effects such as myelosuppression that could result during prolonged treatment with certain antiviral nucleosides. Therefore, the rate of recovery of CD4 cells could be overestimated in the model (Cohen and Fauci, 2000).

Katlama et al. (1996) reported a log(reduction in virus load to  $-0.8 \pm 0.1$  (mean  $\pm$  SEM,  $n = 26$ ) log drop HIV for patents taking ZDV (600 mg/day)



that occurred during the second week of therapy, followed by a steady increase in HIV to below treatment levels. These values are similar to other reports in the literature (Eron et al., 1995; Package insert 1, 2002). The pharmacodynamic model predicted a 0.3 log decrease would occur within the first weeks of treatment followed by a rebound of HIV load to almost baseline after the second week with oscillations to a level slightly lower than baseline by week 4 (curve 1). Considering the implied magnitude in SD ( $S.D. = SEM \times \sqrt{n}$ ), this simulation is within the range of seen in patients.

Montaner et al. (1998), reported a nadir 1.2 log drop in HIV in patients ( $n = 362$ ) taking 300 mg of 3TC per day at week 2 of therapy, followed by a rise to 0.45 logs of pretreatment virus load by week 8. Similar results have been reported by other studies (Eron et al., 1995; Package insert 2, 2002). The model predicted a nadir 1.4 log drop between week 2.5 and 3, with rebound to pretreatment values by week 6, and subsequent fluctuations around baseline between weeks 6 and 12 (curve 2). Montaner et al. (1998), Eron et al., (1995) and Cooper et al. (1997) reported that a combined regimen of ZDV (600 mg/day) and 3TC (300 mg/day) resulted in  $\log_{10}$  (reductions in viral loads (mean  $\pm$  SEM) of  $-1.6 \pm 0.2$  ( $n = 26$ ) and  $-1.6 \pm 0.075$  ( $n = 75$ ), respectively, at week 4, with a rebound in virus load to a pseudo-steady state level of  $-1.0 \pm 0.8$  logs ( $n = 26$ ), and  $-1.0 \pm 0.075$  ( $n = 75$ ), respectively. The model predicted a nadir 1.65 log drop between weeks 3.5 and 4, and a rebound to approximately baseline within 8 weeks of starting treatment (curve 3). Model predictions for CD4 counts were in the range reported in the above clinical trials.

Indinavir monotherapy with 2400 mg/day produces a 1.5 log nadir in HIV loads that occurs approximately 4 week after initiation followed by a rise to 1.25 log of baseline by week 10 (Gulick et al., 2000; Hirsch et al., 1999; Package insert 3, 2002). The model predicted a 2 log nadir at week 2 and 3, followed by a rise to a pseudo-steady state oscillation point of approximately 1 logs below pretreatment levels between weeks 5 and 6 (curve 4). Again, CD4 profiles were similar to those reported in the above clinical trials.

The evolution of standard care for HIV-infected patients followed from single nucleoside to double nucleoside to double nucleoside together with a protease inhibitor (e.g. indinavir) or a non-nucleoside reverse transcription inhibitor (NNRTI) (Rutchmann and Hirschel, 1997). Therefore, it was not surprising that a recent literature search uncovered only one clinical trial that included ZDV together with indinavir without a third drug (Lewi et al., 2000). This trial was terminated early due to the advent of HAART. The published results did not include a plot of HIV loads during therapy to compare with the model prediction (curve 5), although CD4 counts predicted by the model simulations were in agreement with those reported in that partial clinical trial.

The model predicted elimination of virus to below the limit of detection for the combination of 3TC plus indinavir (curve 6), with a  $>6$  log reduction in HIV plasma load at week 12. Therefore, the model would predict achievement of zero virus load for triple drug HAART therapy. The simulation of CD4 counts/ml was terminated at week 12, since HIV loads approached zero at that time. HAART therapy has proven to be very effective, with up to 1/3 of patients maintaining HIV loads  $<500$  copies per ml for over 3 years (Kaufman et al., 2000; Gulick et al., 2000). However, this result is clearly not realistic, since HAART therapy is not curative and recent data demonstrate that certain memory cells are chronically infected with HIV (Ostrowski et al., 1999; Blankson et al., 2002).

This study demonstrated for the first time that pharmacodynamic models that link the single dose pharmacokinetics with cellular pharmacology and cell-free enzyme inhibition studies can predict median trends for clinical trials of HIV drug therapy. Simulated virus loads and CD4 counts versus time and dose were similar to previous reports from large Phase II and III clinical studies with single or dual combinations of nucleoside RT inhibitors and monotherapy regimens with protease inhibitors. Therefore, this model may be used to maximize the information content obtained early in the drug development process to predict the overall efficacy of a potential anti-HIV drug when used alone or in dual combination. Similar

models may be useful in the design of future clinical trials since monotherapy trials with new agents are no longer possible (Fauci et al., 2000).

The model predicts an increase in viral load after reaching a minimum, even in the absence of viral resistance. Havlir et al. (2001), reported that patients treated with indinavir together with ZDV and 3TC who achieved virologic suppression frequently experience intermittent viremia that did not predict subsequent virologic failure and, therefore, did not indicate the development of viral resistance. The predator prey model could be used to rationalize such increases. Briefly, productively infected cells continually produce HIV throughout their survival (median survival = 2.6 days) and the median survival of uninfected cells is substantially longer (100 days). At the start of drug therapy, the number of uninfected CD4 cells remains depleted due to the infection of cells in the blood. During treatment the production of infected cells (median survival = 2.6 days) is substantially inhibited by the RT inhibitors and the majority CD4 cells remain uninfected (median survival = 100 days). Due to input of new cells into the circulation the CD4 cell population increases with time. Unless the treatment protects all CD4 cells from infection, a proportion of uninfected cells will serve as target cells for the virus. As the total number of CD4 cells in the blood increases with time, and assuming a constant proportion of target cells, the total number of CD4 cells susceptible to infection will also increase in number. The model assumes that new infections are proportional to the product of virus load and susceptible CD4 cells. Thus, the product  $V \times \text{CD4}$  should increase with time. This would correspond to an increase in both viral load and CD4 counts. Since the total number of CD4 cells is limited by the blood volume and cell counts may not exceed  $\sim 1.5 \times 10^3$  cells per  $\mu\text{l}$ , CD4 and virus load should reach a new equilibrium (Nelson et al., 2000; Ramratnam et al., 2000; Stafford et al., 2000).

With the advent of HAART, there is insufficient clinical data available in the literature to assess the ability of the model to predict the efficacy of dual combinations of nucleoside RT inhibitors with protease inhibitors. New variations of combination HAART therapy are being developed in

which one agent is replaced with a novel agent of the same class. One could speculate that a TK dependent RT inhibitor, (e.g. ZDV) may be replaced with D4T, or a thymidine independent RT inhibitor, (e.g. 3TC), may be replaced with ddI or abacavir. A protease inhibitor (e.g. indinavir) may be replaced with a newer protease inhibitor such as nelfinavir or Kaletra (lopinavir/ritonavir). Provided the efficacy of the individual agents can be reliably predicted independently, it may be reasonable to expect that substitution of an existing agent in HAART with a novel drug of the same class at a dose regimen predicted to produce the same monotherapeutic efficacy as the agent replaced should result in a HAART of similar potency.

This model does not consider drug interactions, which could markedly alter the efficacy of potential dose regimens. These interactions may result in improved efficacy, e.g. ritonavir inhibits hepatic metabolism of indinavir, resulting in a larger plasma AUC value and with a concomitant increase in toxicity (Solas et al., 2002; van Heeswijk et al., 1999). A larger AUC may also result in a greater antiviral effect than could be predicted from pharmacokinetic data measured in the absence of ritonavir. Conversely, some interactions may produce lower than expected efficacy, e.g. Saquinavir concentrations may be significantly lowered in regimens containing the combination of delavirdine plus adefovir dipivoxil compared with regimens containing delavirdine alone (Fletcher et al., 2000).

The model predicted that a HAART combination of ZDV with lamivudine and indinavir would produce a greater than  $6 \log_{10}(\text{HIV load})$  reduction, corresponding to a virtual complete virus elimination within 5 week. This is clearly greater than that actually achievable with HAART. Possible reasons for model overestimation for these more efficacious drug regimens may include the fact that not all tissues harboring HIV are necessarily exposed to equal concentrations of active drug. ZDV, 3TC, and indinavir may not partition equally among all infected tissues including the plasma, lymph nodes and tissues behind the blood–brain barrier (Brack-Werner, 1999; Cavert et al., 1997; Haas et al., 2000; Hlavacek

et al., 2000). Furthermore, ZDV and 3TC are not dependent on the same kinases to form the active nucleotide, so that even if the nucleoside penetrated all tissues equally, NTP would not necessarily be present to equal degrees in all tissues. Moreover, the model only considers actively viral-replicating infected CD4 cells and not latently infected cells, dendritic cells, astrocytes or other tissues that have the potential to perpetuate the infection (Brack-Werner, 1999; Cavert et al., 1997; Haas et al., 2000; Hlavacek et al., 2000; Nelson et al., 2000; Ramratnam et al., 2000). Taken together, the model is predictive for therapy with single or dual combinations of nucleoside RT and protease inhibitors. The degree of viral suppression achievable with HAART warrants the development of more complex pharmacodynamic models that could incorporate non-uniform drug disposition and/or including latently infected CD4 cells, dendritic cells and other cells that have the potential to perpetuate the HIV infection.

## Acknowledgements

This work was supported in part by Public Health Service grant 1RO37-AI-41980, Emory University Center for AIDS Research grant 1P30-AI42847 and the US Department of Veterans Affairs. This work was presented in part at HIV DART 2000, Frontiers in Drug Development for Antiretroviral Therapies. Isla Verde, Puerto Rico. December 17–21, 2000. Abstract #086. The authors wish to acknowledge Dr Paul Black of QuadPharma Inc and Dr Prem L. Sharma of Emory University School of Medicine for helpful discussions, and Monica L. Hurwitz for designing Fig. 1.

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